

**MASS REARING TECHNIQUE FOR MANGO PULP WEEVIL,
Sternochetus frigidus (Fabr.) (COLEOPTERA: CURCULIONIDAE)**

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ABSTRACT

A mass rearing technique for mango pulp weevils (MPW) was developed in Brooke's Point, Palawan that ensured the supply of test insects at different stages of development for irradiation trials. The mass rearing technique synchronized chicken-egg-size mango host fruits (65 days after flower induction) enclosed in nylon bags, grown on trees with the release of male and gravid female weevils inside the nylon bags for oviposition. This technique produced a mean number of 90.3 ± 16.2 larvae (5th instar) grown from 100 pieces 'carabao' mango fruits. A mean number of 63.0 ± 22.5 and 180.3 ± 21.4 of pupae and adult MPW respectively, were produced from 100 pieces 'carabao' mango fruits in separate trials.

Key words: insect rearing technique, 'carabao' mango

INTRODUCTION

Mango pulp weevil (MPW), *Sternochetus frigidus* (Fabr), is a major constraint in the export of fresh mango fruit. It is found only in southern Palawan, an island in southern Philippines and probably introduced from Borneo (Basio *et al.*, 1994). The pest has prevented mango exports from the Philippines to the U.S. mainland except those sourced from Guimaras Island that is certified pulp weevil-free.

The Palawan Island group has been placed under quarantine since 1987 in order to prevent the spread of the pest to other mango growing areas in the country. A Special Quarantine Administrative Order specifies that the movement, transfer or carrying of mango plants, fruits or parts from Palawan is prohibited.

This pest feeds and develops inside the fruit but it shows no visible signs of damage on the fruit peel that would indicate its presence. Eggs are laid singly on the fruit peel and the emerging larvae bore into the fruit peel, feed and develop on the pulp. The duration for the development of the 1st instar larva is about 6.4 days. Development from the 2nd to the 5th instar larva averages 13.9 days. The 5th instar larva is voracious, makes a 1-cm diameter hole oftentimes in the midsection of the fruit close to the seed. It confines itself into the hole until frass accumulates in the hole, turning it into a dry chamber or pupal cell. The prepupa is quiescent while the pupa is exarate and active. The adult remains inside the pupal cell still inside the fruit until the latter is rotten. Total development of MPW in 'carabao' mango from egg to adult stage is 32 days with 5 larval instars (De Jesus and Gabo, 2000).

In backyards in Brooke's Point, southern Palawan infestation of trees was as high as 43% (De Jesus and Cortez, 1998). An Integrated Pest Management program developed involved the use of control strategies, namely, cultural methods (open-center pruning and sanitation), pest monitoring and chemical control (Medina *et al.*, 2005).

The mango fruit industry can be expanded thru the development of quarantine treatments to permit exports. Irradiation is a viable alternative to disinfest mangoes of MPW. It does not leave toxic residues and does not make food harmful to human health (WHO, 1994). It is an accepted quarantine treatment Hawaii for instance uses radiation treatments to export 5,000-6,000 tons of tropical fruits and vegetables to the United States mainland annually (Follett, 2009).

The protocol for irradiation treatments requires scientific evidence before it can be established which in turn need large volume of test insects in order to run the tests. However, no artificial diet exists to culture the insect in the laboratory for the production of this insect in large volumes. Moreover, the only facility for irradiation in the country is located in the Luzon mainland so that the test insects need to be airlifted for the irradiation trials. As such a mass-rearing technique for MPW needed to be developed to produce test insects at different stages of development that can be transported to an irradiation facility for research purposes.

This study was conducted to establish a mass rearing technique for the 5th instar larvae, pupae and adults of MPW for irradiation trials.

MATERIALS AND METHODS

Rearing Conditions

Infested 'carabao' mango fruits were collected from trees in the field in Brooke's Point, Palawan. Emerging adults were sexed following De Jesus *et al.* (2002) and a weevil stock culture was established in the MPW Research Laboratory also located in Brooke's Point. Weevil stock culture were reared in several 5 x 6" round plastic jars covered with nylon cloth and lined with white flat napkins. Small pieces measuring 15 x 15 x 15 mm cut from 70-100 day-old green mango fruits were provided daily serving as food (Fig. 1). After 24 h, the adults were transferred to fresh jars and green mango substrates (De Jesus and Gabo, 2000).



Fig. 1. Adult MPW stock culture reared in round plastic jars with nylon cloth cover.

To rear large number of weevils most efficiently green mango substrates were always made available by maintaining 13 mango trees (20-yr old) in the laboratory compound with fruits all year round. A staggered chemical induction of mango trees using 1.5% KNO_3 was done. Insecticides were applied only up to the 37th days after flower induction (DAFI) to control the leafhopper *Idioscopus clypealis*. At about 70-100 DAFI green mango fruits were collected daily and used as substrates in the laboratory.

Preparation for Oviposition

Ten pairs of male and female weevils (3-wk old) were placed in small plastic jars and reared in the same manner as the stock culture. A small piece measuring 15 x 15 x 15 mm cut from 70-100 day old green mango fruits were provided daily serving as food (Fig. 2). To determine if the females were gravid the 24-hr-old substrates were gathered and individually examined under the microscope to find eggs (De Jesus and Gabo, 2000; De Jesus, 2008). Rearing cups with gravid females were labeled in preparation for field transfer and oviposition on developing mango fruits.

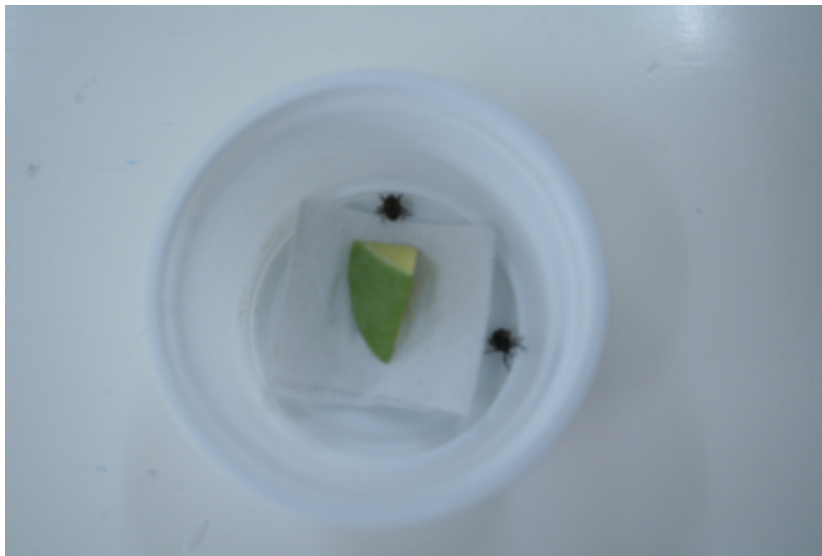


Fig. 2. Paired male and female MPW for mating and oviposition.

Oviposition and Field Rearing of Weevils

The mass rearing of MPW at the 5th instar larval stage, pupae and adult stages was done in the field at Brooke's Point.

Thirty-five mango trees (15-20 yr old) were induced to flower using KNO_3 on a staggered basis to provide enough developing mango fruits for weevil oviposition. Mango fruits at 65 DAFI or approximately at chicken-egg-size (6 cm length) fruit stage in the mango trees were individually enclosed in nylon bags. A pair of male and gravid female weevils reared in the laboratory was then released into each bag for oviposition. The nylon bags were tagged indicating date of MPW release into the bag.

After 48 hours the weevils were removed and the mango fruits remained enclosed in nylon bags. While the mango fruits develop on mango trees the weevils also develop at the same time

inside mango fruits. At 115 DAFI the weevils have grown into adults and the fruits at green mature stage were also ready for harvesting.

In the rearing for 5th instar larvae and pupae the schedule of release of the pair of gravid female and male weevils into the nylon bags was also at 65 DAFI but harvesting was timed earlier at 95 and 100 DAFI, respectively. The harvesting schedule was based on life history data from the study of De Jesus and Gabo (2000).

To ascertain that the developing weevils have grown into 5th instar larvae and pupae mango fruit samples were collected and dissected following the method of De Jesus and Gabo (2000).

Fruit Collecting, Transporting and Dissection

The Philippine Nuclear Research Institute (PNRI) of the Department of Science and Technology in Diliman, Quezon City operates a Multipurpose Gamma Irradiation Facility. The mango fruits with MPW at the desired stage of development were collected and sent to PNRI for irradiation.

The fruits were collected by individually cutting with the use of a pruning shear at the pedicel leaving at least 1 cm of pedicel on the fruit for minimum latex flow. The fruits were brought to the MPW Research Laboratory for packing. Fruits were packed in carton boxes containing 20 pieces per box and transported by plane to PNRI at Quezon City at the following day.

One hundred fruits with weevils were sent per shipment 3 shipments each for 5th instar larva, pupa and adults. Shipments of mango fruits with weevils were allowed only after securing a special permit to transport from the Bureau of Plant Industry for research purposes. The entire process of fruit collecting, packing and transporting were all guarded by a Plant Quarantine Officer.

At PNRI the mango fruits were cut-open and actual counts of MPW found inside the fruits at all stages of development were recorded upon arrival for the shipments of 5th instar larvae and pupae. For shipments of adults counts were made 2 days after arrival.

RESULTS AND DISCUSSION

The average number of 5th instar larvae and other developmental stages of MPW grown from 100 pieces 'carabao' mango fruits up to 95 DAFI by field mass rearing technique is shown in Table 1. An average number of 90.3 ± 16.2 larvae constituted 78% of the total 115.7 ± 33.9 weevils that developed. MPW infestation on mango fruits was 44.7%. The 5th instar larvae developed into adults in the laboratory with a sex ratio of 147:152 (male:female).

In mass rearing for pupae an average number of 63.0 ± 22.5 pupae was produced from 100 pieces 'carabao' mango fruits harvested at 100 DAFI. This consisted of 42.8% of the total of 147.3 ± 78.6 weevils that developed (Table 2). MPW infestation on mango fruits was 44 %. The pupae were able to develop into adults in the laboratory with a sex ratio of 155:145 (male:female).

The average number of adult MPW grown from 100 mango fruits was 180.3 ± 21.4 (Table 3). This comprised 92.2 % of the total 191.3 ± 18.9 weevils that developed. MPW infestation on mango fruits was 49.7%. Sex ratio of adults was 153:147 (male:female).

Table 1. Average number of 5th instar larvae and other developmental stages of MPW grown from ‘carabao’ mango fruits up to 95 DAFI by mass rearing technique*.

Parameter	Average Number + SD	Range
Total number of weevils reared	115.7±33.9	104-134
% Infested fruit	44.7±2.0	43-47
Total number of larvae per fruit		
3rd instar	0.7±1.2	0-2
4 th instar	11.0±5.6	6-17
5 th instar	90.3±16.2	81-109
Pre-pupae	1.0±1.0	1-2
Number of pupae	8.7±4.7	5-14
Number of adults	4.0±5.3	2-10
Adult sex ratio	147:152	47-54(male)
(Developed from larvae)	(male:female)	46-53(female)

*Data obtained from dissection of 100 mangoes per replicate with 3 replications.

Table 2. Average number of pupae and other developmental stages of MPW grown from ‘carabao’ mango fruits up to 100 DAFI by mass rearing technique*.

Parameter	Average Number + SD	Range
Total number of weevils reared	147.3±78.6	93-189
% Infested fruit	44.0±5.3	38-46
Total number of larvae per fruit		
3rd instar	0	-
4 th instar	0	-
5 th instar	60.67±40.6	14-88
Pre-pupa	0	
Number of pupae	63.0±22.5	40-85
Number of adults	23.7±15.5	8-39
Adult sex ratio	155:145	50-53(male)
(Developed from pupae)	(male:female)	47-50(female)

*Data obtained from dissection of 100 mangoes per replicate with 3 replications.

MPW is difficult to mass rear because of the absence of an artificial diet. De Jesus (2008) suggested that the key to mass rear the weevil is to synchronize the peak of egg lay and the right stage of mango fruit for oviposition thus it will be appropriate that rearing of weevils be done in the field using developing mango fruits. It is important to understand that the weevil prefers to oviposit at 61-90 DAFI and the weevil peak of oviposition is at 6-18 wk after female adult emergence (De Jesus, 2008; De Jesus and Cortez, 1998).

Table 3. Average number of adult and other developmental stages of MPW grown from 'carabao' mango fruits up to 115 DAFI by mass rearing technique*.

Parameter	Average Number \pm SD	Range
Total number of weevils reared	191.3 \pm 18.9	175-212
% Infested fruit	49.7 \pm 7.8	41-56
Total number of larvae per fruit		
3rd instar	0	-
4 th instar	0	-
5 th instar	3.3 \pm 1.2	2-4
Number of pre-pupa	1.0 \pm 0	1-1
Number of pupae	6.7 \pm 8.1	2-16
Number of adults	180.3 \pm 21.4	168-205
Adult sex ratio	153:147	50-52(male)
	(male:female)	49-50(female)

*Data obtained from dissection of 100 mangoes per replicate with 3 replications.

Following that suggestion the mango host plant was used to mass rear MPW. And as a follow on the timing of chemical flower induction of mango trees was synchronized with the rearing of weevils in the laboratory so that the mango fruits were 65 DAFI (within the range of the weevil preference for oviposition) when the female weevils became gravid. With this method the oviposition of MPW on mango fruits in the field became successful and its development inside mango fruits were sustained. The rearing technique developed was successful, however, it was not very easy to do. The process of synchronizing chemical flower induction of mango trees with the individual tagging of mango fruits using nylon bags and the actual release of gravid females in the field was very laborious and costly. The potential benefits that could be gained from the irradiation trials were taken into consideration so that these constraints were set aside.

In other curculionids, successful mass rearing techniques also involve the use of host plants. The sitona weevil, *Sitona humeralis* Stephens is reared on potted lucerne plants (Sue *et al.*, 1980); sweet clover weevil, *Sitona cylindricollis* Fahr is fed with cut sweet clover leaves (Hans, 1961); clover root curculio, *Sitona hispidus* (F.) is grown on legumes (Byers, 1995); sweetpotato weevil, *Cylas formicarius* Summers used pureed sweetpotato storage roots (Kays *et al.*, 1993).

CONCLUSION

A mass rearing technique was developed for MPW. This technique synchronized chemical flower induction of mango trees to produce 65 DAFI mango host fruits enclosed in nylon bags, grown on trees with the release of male and gravid female weevils reared in the laboratory into the nylon bags for oviposition. This produced an average number of 90.3 \pm 16.2 larvae (5th instar) grown from 100 pieces 'carabao' mango fruits. An average number of 63.0 \pm 22.5 pupae from 100 pieces 'carabao' mango fruits and 180.3 \pm 21.4 adults also from 100 pieces 'carabao' mango fruits were also produced in separate trials.

This is the first mass rearing technique developed for MPW. With this method the supply of 5th instar larvae, pupae and adult MPW test insects in large volumes needed for irradiation trials can be produced.

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